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Amino Acid Supplementation of Opaque-2 Corn for Growing Pigs

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CORN FOR GROWING PIGS

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable as meeting the thesis requirements for this degree, but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Thesis Adviser   Date

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INTRODUCTION

Protein needs and amino acid requirements are of major interest in the swine industry today. A considerable amount of research has been conducted on these subjects and more is sure to follow. Amino acid content and balance are now being recognized as more important factors in swine nutrition than total protein alone.

Recently, the development of opaque-2 corn with higher amounts of lysine and tryptophan than normal corn has stimulated interest in its presence in simplified diets to supply the amino acid needs of swine. Performance of pigs fed opaque-2 corn has generally been superior to that of pigs fed normal corn. Although opaque-2 corn is inadequate as the only source of amino acids in a growing pig diet, it may, with proper supplementation, prove to be a practical and economical base for swine diets. Data suggest that pigs require less supplemental protein when fed opaque-2 corn than when fed normal corn.

The purpose of the research presented herein was to study the adequacy of the essential amino acids lysine, methionine, tryptophan, threonine, and isoleucine in opaque-2 corn diets for young pigs. Rate of gain, feed efficiency, and plasma amino acid levels were criteria used to evaluate the diets.

REVIEW OF LITERATURE

Value of Opaque-2 Corn

The influence of adding lysine to normal corn diets for growing pigs was demonstrated by Magruder, Sherman and Reynolds (1961). These workers supplemented 14% and 16% protein corn-soybean meal diets with 0.1% lysine and found pigs fed with the 16% protein diet with 0.1% lysine addition had the highest gains. The 14% protein diet with 0.1% lysine added was equal to the 16% protein diet in promoting gain. Nielson et al. (1963) found that lysine supplementation of 10% and 12% protein corn-soybean meal finishing diets improved performance of growing pigs. The improved performance with lysine supplementation would indicate that lysine is limiting not only in corn, but in a corn-soybean meal diet as well.

Some research has indicated that lysine may not be the one most limiting amino acid in corn. Tryptophan has been mentioned as well. Gallo, Pond, and Logomarsino (1968) found that additions of lysine, threonine, isoleucine, and methionine to normal corn resulted in significantly lower gains than lysine and tryptophan additions alone. This would indicate that tryptophan is very limiting. Studies with normal corn rations by Gallo and Pond (1968) showed that response to lysine supplementation was obtained only with the simultaneous addition of tryptophan.

Baker et al. (1969), using a fortified corn diet, showed that 0.2% lysine addition to the diet caused lower gain and feed intake. When 0.5% DL-tryptophan was added as well, the depression caused by lysine was overcome. Methionine supplementation at 0.1% did not affect performance of the pigs when it was added to lysine or lysine-tryptophan supplemented corn. From the data the authors suggested that tryptophan was the most limiting amino acid, lysine the second and that methionine was not the third limiting in normal corn.

These reports, along with numerous others, would indicate that either lysine or tryptophan (probably lysine) is the most limiting amino acid in normal corn when considering swine diets. It was of considerable interest when Mertz, Bates and Nelson (1964) reported a different amino acid pattern in a strain of corn known as opaque-2 corn. Opaque-2 corn was discovered to contain 69% more lysine than normal corn. Several other amino acids including tryptophan were present in higher amounts also. The higher lysine content was reportedly due to: (1) increased lysine in the acid soluble fraction, (2) increased lysine in the zein fraction and (3) a reduction in the ratio of zein to glutelin. Mertz et al. (1965) found that opaque-2 corn was superior to normal corn as a component in a rat diet.

Cromwell, Pickett and Beeson (1967) studied opaque-2 corn to see if improved performance was due to an increased lysine content alone. Amino acid analysis showed opaque-2 corn was higher in all essential amino acids except leucine on a percent of air dry basis. The largest increases were a 100% increase in lysine and a 60% increase in tryptophan. Pigs ranging from 10 to 22 kilograms initially were fed these corns and showed significantly higher gains when opaque-2 corn was substituted for normal corn in diets suboptimum in protein.

Sihombing, Cromwell and Hays (1969) also reported improved gains and feed utilization in pigs fed opaque-2 corn rather than normal corn. Again opaque-2 corn was more beneficial in lower protein diets. Apparent digestibility of fat was significantly lower in diets containing opaque-2 corn while apparent digestibility of dry matter, crude fiber, ash and nitrogen free extract was not consistently affected by corn source.

Veum *et al*. (1973) compared opaque-2 corn and normal corn as amino acid sources for growing-finishing swine. Pigs were fed from 26 to 94 kilograms. Those fed diets containing normal corn and soybean meal (14% protein) had greater average daily gains than the pigs fed opaque-2 corn-soybean meal diets. The normal corn-soybean meal diet in this case contained more lysine. However, when pigs were fed opaque-2 corn supplemented with lysine, methionine and tryptophan they gained faster than those receiving normal corn with lysine, methionine and tryptophan supplementation. Both corns were supplemented to meet National Research Council (NRC) recommendations for the three supplemented amino acids. This again would indicate an advantage for opaque-2 corn in diets suboptimum in total amino acid level. Amino acid analysis indicated that the difference in lysine and tryptophan content between opaque-2 and normal corn was not as great in this study as in other research. Marroquin, Cromwell and Hays (1973) noted differences in varieties of opaque-2 corn with one variety analyzing 0.37% lysine and the other 0.45% lysine. With either variety, diets containing opaque-2 corn gave increased gains and improved feed efficiency. The apparent digestibility of protein was significantly greater in diets containing opaque-2 corn as well. This is in agreement with Cromwell et al. (1969), who also found greater nitrogen retention in opaque-2 corn diets.

Klein et al. (1971) compared opaque-2 corn and normal corn by supplementing normal corn with six amino acids which he named as the most limiting in normal corn. These six amino acids; lysine, tryptophan, valine, isoleucine, threonine and methionine were added so that the levels of the six were equal to the levels of opaque-2 corn. A response was shown to supplementation in all trials but performance was still inferior to opaque-2 corn, indicating additional amino acid or nutrient components in the opaque-2 corn. Supplementation of an additional 0.1% lysine in the amino acid supplemented corn diet gave significantly better gains, indicating that lysine was still limiting in the normal corn diet. Other results indicated that lysine and tryptophan are the major factors influencing the superiority of opaque-2 corn. Similar findings were reported by Gipp and Cline (1972).

The ability of opaque-2 corn diets to give normal gains with less protein supplement than normal corn is the basis for its economical importance. Drews et al. (1969) reported that opaque-2 corn diets fed to chicks and pigs resulted in gains equal to those obtained from a normal corn-soybean meal diet which was 5% higher in soybean meal. Data recorded by Gipp and Cline (1972) showed similar results. In this experiment, pigs fed opaque-2 corn (10.8% protein) with or without soybean meal supplementation had equal gains to pigs fed corn-soybean meal diets with higher protein (12%).

Growth performance of finishing swine fed opaque-2 corn diets supplemented with 0.15% lysine was equal or superior to that of pigs fed a 12% protein normal corn-soybean diet (Jensen et al., 1967).

Oestemer et al. (1970) conducted three trials to determine whether opaque-2 corn contained adequate dietary methionine for the pig. Opaque-2 corns containing 0.275, 0.279 and 0.227% methionine plus cystine were supplemented with 0.07, 0.14, 0.21 or 0.28% DL-methionine. No significant improvement in performance at any level of supplementation was attained. These data may suggest that methionine may not be as limiting in opaque-2 corn as some researchers thought or methionine requirements may be lower than those published by N.R.C. (1968).

Limited research has been conducted with opaque-2 corn supplemented with protein sources other than soybean meal. Thomas and Kornegy (1972) found that high lysine corn-peanut meal diets resulted in higher average daily gains and improved feed utilization over normal corn-peanut meal diets. Further performance increases were obtained by supplementing either diet with lysine. Rate of gain and feed efficiency were positively related to the lysine content of the diets irrespective of the kind of corn up to a level of about 0.85% total lysine. They concluded that in a 16% protein high lysine corn-peanut meal diet the lysine content of high lysine corn is still not adequate to overcome the lysine deficiency.

Amino Acid Requirements of Growing Swine

Lysine. A large amount of research has been undertaken in recent years in an attempt to determine the amino acid needs of swine. Using skim milk and sesame oil meal as protein supplements to cerelose and corn starch, Hutchinson et al. (1957b) suggested that the L-lysine requirement for pigs weighing 4.3 kg was $<$ 0.935% of the 14.25% protein diet. L-lysine made up 6.56% of the total protein. Mitchell et al. (1965) found lysine levels of 1.2 to 1.34% (about 5.9% of the total protein) were required when 5 kg pigs were fed diets of 22% protein.

Studies by Germann, Mertz and Beeson (1958) with weanling pigs weighing about 11 kg showed maximum gains and efficiency at 0.62% dietary lysine levels. Female pigs appeared to need no more than 0.52% lysine. Hutchinson et al. (1957a) used an 11.69% crude protein diet with varying lysine levels to evaluate the lysine needs in pigs weighing 16 to 25 kilograms. Total lysine levels involved were 0.32, 0.42, 0.52, 0.62, 0.72 and 0.82%. The levels of 0.32 and 0.42% lysine were inadequate while the 0.52 to 0.82% levels gave similar results. Conclusions were that 0.52% L-lysine (4.44% of the dietary protein) was adequate for pigs of this size. McWard et al. (1959) showed the effects of lysine level on 14 kg pigs fed 12.8 and 21.7% protein diets. At the 12.8% protein level the authors suggested 0.71% lysine (5.55% of the total protein) and at the 21.7% protein 0.95% lysine (4.38% of the protein) was recommended. N.R.C. (1968) recommends 1.2 and 0.7% lysine for pigs weighing 5 to 10 kg and 20 to 35 kg, respectively. These levels would be equivalent to 5.45 and 4.4% of the total protein.

Methionine. Curtin et al. (1952_a) attempted to determine the methionine plus cystine requirement of 16 kg pigs fed a diet containing 22% protein from isolated soybean protein and brewer's dried yeast. The results showed the requirement to be about 0.7% of the diet or 3.2% of the protein. The authors felt that cystine could be used to replace methionine in the ration up to 1.7% of the protein. Using a 22% protein soybean meal-glucose diet, Curtin et al. (1952_b) reported the methionine requirements to be $<$ 0.31% when the ration contained 0.38% cystine.

Kroening, Pond and Loosli (1965) sought to obtain the sulfur amino acid requirements of baby pigs 2 to 7 weeks old. The results indicated a methionine-cystine need of 0.5, 0.6, and 0.7% at 12, 18 and 25% protein, respectively.

A methionine-cystine requirement of approximately 0.42% (3.33% of the dietary protein) was reported by Becker et al. (1955_a) when using a 12.6% protein diet. Indications were that the D-isomer of methionine was equal to the natural isomer in activity. They also suggested that cystine could apparently satisfy 40% of the total requirement of methionine which is in agreement with N.R.C. (1968).

N.R.C. (1968) lists the methionine needs at 0.8 and 0.5% for 5 to 10 kg and 20 to 35 kg pigs, respectively. These percentages correspond to 3.46 and 3.13% of the dietary protein recommended. It is questionable as to whether or not these values are accurate since it would be impossible to obtain a dietary level of 0.8% methionine with a practical corn-soybean meal diet and very difficult to achieve a 0.5% level, especially at recommended protein levels. This is the case even when it is assumed that cystine can provide 40% of the needed methionine.

Tryptophan. Becker *et al*. (1955_b) conducted research with pigs weighing 13.6 kg fed a corn-fish meal diet. With adequate nicotinic acid, the L-tryptophan requirement was determined to be 0.115% in a 15.3% protein diet.

The tryptophan requirement of early weaned pigs from 3 to 7 weeks of age was studied by Gallo and Pond (1966). Nicotinic acid was added at levels equal to or greater than N.R.C. recommendations since tryptophan may be converted to the vitamin nicotinic acid if the vitamin is deficient. Levels of 0.18 and 0.22% tryptophan gave maximum gains in rations of 16 and 20% protein, respectively. These levels account for approximately 1.1% of the total protein.

Boomgaardt and Baker (1973) fed 9 to 11 kg pigs corn-gelatin diets supplemented to be adequate in all amino acids except tryptophan. They found minimum requirements to be 0.71, 0.67 and 0.66% tryptophan when expressed as a percent of the dietary protein levels of 10, 14 and 18% protein, respectively. Expressed as a percent of the diet, minimum tryptophan requirements rose from 0.071 to 0.119% tryptophan as protein levels increased from 10 to 18%.

N.R.C. (1968) recommends 0.18 and 0.13% tryptophan for pigs that weigh 5 to 10 kg and 20 to 35 kg, respectively. These levels approximate 0.8% of the dietary protein.

Threonine. Maximum gains and feed efficiency were obtained from rations containing 0.4% L-threonine (3% of crude protein) in studies by Beeson, Jackson and Mertz (1953). Semi-purified diets with corn as a base were fed to 11 kg pigs with levels of L-threonine at 0.2, 0.4, 0.5, 0.6 and 0.7% of the diets in this experiment.

Kroening et al. (1965) obtained data from pigs 2 to 7 weeks of age which indicated a dietary requirement of about 0.63% threonine. Becker, Ullrey and Terrill (1954) suggested levels of 1.1% threonine for pigs from 1 to 4 weeks of age fed a 22% protein diet and 0.61% threonine for pigs 5 to 9 weeks of age fed a 12% protein diet. These two levels of threonine account for about 5% of the protein in their respective diets. Data presented by Mitchell et al. (1968a) would indicate a requirement of 0.6% threonine for pigs at 10 kilograms.

N.R.C. (1968) recommends 0.70% and 0.45% threonine for 5 to 10 kg and 20 to 35 kg pigs, respectively. These levels correspond to 3.2% and 2.8% of the total protein.

Isoleucine. An early attempt to find the isoleucine requirement of the weanling pig was reported by Brinegar et al. (1950). Twenty-two percent protein diets containing blood flour and methionine were fed. Maximum gain was derived from levels of 0.7% isoleucine (3.2% of the dietary protein).

Becker et al. (1957) fed diets containing 13.35% and 26.7% protein to weanling pigs. Data indicated requirements of 0.46% isoleucine (3.4% of the total protein) and 0.65% isoleucine (2.4% of the total protein) at the two levels of protein, respectively.

Earlier, Becker et al. (1954) recommended levels of 1.27% and 0.74% isoleucine for pigs 1 to 4 weeks old and 5 to 9 weeks old, respectively. Becker et al. (1963) used a semi-synthetic diet containing 22% protein as a ration for pigs of 5 kg and achieved maximum rate and efficiency of gain at 0.76% isoleucine (3.45% of the dietary protein).

Oestemer, Hanson and Meade (1973) fed diets containing adequate but not excessive amounts of all amino acids other than isoleucine. Predicted requirements using gain, feed efficiency and protein efficiency ratio as criteria of evaluation were 0.52, 0.48 and 0.45% of the diet, respectively.

N.R.C. (1968) recommends isoleucine levels of 0.76% and 0.50% for 5 to 10 kg and 20 to 35 kg pigs, respectively.

The inconsistency in the amino acid requirements obtained in the previous literature perhaps was due to one or more of the following: variation in composition of feedstuffs or diet ingredients, differences in protein quality in feed, availability of amino acids, lean to fat ratio of pigs, sex, genetic background, weight and methods of diet analysis. It is therefore necessary to report as much of these data as possible since one or several variations may affect the required dietary levels.

Plasma Free Amino Acid Studies

Mitchell et al. (1968b) sought to determine if plasma-free amino acids could be used to accurately evaluate dietary amino acid needs of swine. This study was based on previous work with the rat and chick which indicated that plasma-free amino acid levels remained low until requirements were met in the diet at which time a rapid and linear increase of plasma levels occurred as dietary amino acid levels increased. Conclusions from this study indicated that addition of an amino acid to a diet deficient in that amino acid does not cause a significant increase in the plasma concentration at dietary levels less than the dietary need. In contrast when super-optimal levels of an amino acid are present in a diet, the plasma-free amino acid levels increase.

Zimmerman and Scott (1965) in work with the chick stated two important points. First, it appears that when growth of a young animal is restricted by an amino acid deficiency, the need of tissues for that amino acid tends to maintain that nutrient at a minimum level in the blood. Secondly, they showed that there is a definite relationship between plasma amino acid levels and the amino acid adequacy of the diet. The first limiting amino acid remains at a very low level in the blood irrespective of the amino acid deficiency. When lysine requirements were estimated at 0.83% of the diet, lysine accumulations did not rapidly occur in the blood until a level of 0.94% lysine was attained. In general, lysine accumulated when the diet contained about 10% higher lysine levels than was necessary for maximum growth.

McLaughlin and Illman (1967) using rats found an almost linear relationship between free lysine in the plasma and the dietary level of lysine. Tryptophan, threonine and isoleucine curves also showed a general increase in their plasma content as dietary amount increased.

Harker, Allen and Clark (1968) in a somewhat similar study fed amino acids at four levels: 115, 100, 85, and 70% of the minimum requirements. Lysine and threonine plasma levels were directly related to intake at 4 and 3 levels, respectively. Methionine levels tended to decrease as dietary amounts decreased, while isoleucine and tryptophan were not consistently affected.

Morrison, Middleton and McLaughlin (1961) in plasma studies with rats cited several interesting findings. After an initial lag, plasma amino acid levels rose rapidly in response to added dietary lysine. This rapid response occurred at dietary levels from 0.7% to 1.0% before leveling off. Maximum gain was achieved at 0.8% lysine. They also found that sex significantly influenced plasma lysine levels with females having the higher level. Increased lysine in the diet caused increased lysine in the plasma and resulted in a decrease in plasma threonine. Due to this reciprocal relation, the authors mentioned the possibility of using a lysine-threonine ratio as an indication of diet lysine adequacy. In this study, rate of growth did not significantly influence lysine needs.

It would appear from the literature cited that dietary levels of amino acids may in some way affect the levels of these amino acids in the plasma. There are, however, discrepancies as to the conditions under which an effect results, e.g., dietary level of a particular amino acid in relation to requirements of the animal. Apparently more research is needed in this area.

MATERIALS AND METHODS

Eighty-four crossbred pigs from the University swine herd were randomly assigned by weight, sex and sire to three replications with seven treatments in each. All pens contained four pigs, two barrows and two gilts, averaging about 12.7 kilograms. The study was conducted over a period of 7 weeks from January 3, 1973 to February 21, 1973. The three replications began at one week intervals to insure adequate pig numbers. Each replicate concluded after 5 weeks of study.

Experimental animals were housed in 2.4 by 3.0 meter indoor pens at all times. Pen floors were solid cement and bedded with straw. Similar self-feeders and automatic waterers were provided in each pen. Temperature was maintained at approximately 15 Centigrade.

All diets were mixed at the University feed unit in a twin spiral vertical mixer. Vitamin premixes, mineral mixtures and amino acids were preweighed and mixed with a diluent before being added to the other ingredients in the mixer. Diets were finely ground. Compositions of the diets are shown in table 1. Table 2 presents the proximate chemical analysis of the diets while tables 3 and 4 show the essential amino acid content for the diets.

All pigs were weighed at one week intervals throughout the experimental period. Feed was weighed back at two week intervals and feed efficiency was calculated at those times.

Blood samples of approximately 15 ml were obtained from the anterior vena cava of each pig one day after the 5 week feeding trial ended. Experimental animals were fasted for 12 hours, allowed to

TABLE 1

COMPOSITION OF DIETS (%)

Ingredient	Treatment 1	2	3	4	5	6	7
Opaque-2 corn	96.2	95.8	95.5	95.5	95.4	95.2	
Ground yellow corn							69.5
Soybean meal (44%)							26.7
Dicalcium phosphate[a]	1.4	1.4	1.4	1.4	1.4	1.4	1.4
Ground limestone[b]	1.1	1.1	1.1	1.1	1.1	1.1	1.1
Trace mineralized salt[c]	.5	.5	.5	.5	.5	.5	.5
Premix[d]	.8	.8	.8	.8	.8	.8	.8
L-lysine		.4	.4	.4	.4	.4	
DL-methionine			.25	.25	.25	.25	
DL-tryptophan				.05	.05	.05	
DL-threonine					.15	.15	
DL-isoleucine						.15	

[a] 24% calcium and 21% phosphorus.

[b] 39% calcium.

[c] Contained: NaCl, 97%; Zn, 0.8%; Co, 0.002%; Mn, 0.4%; Cu, 0.48%; Fe, 0.33%; I_2, 0.0011%.

[d] Provided per kg of diet: 3,380 IU of vitamin A, 780 IU of vitamin D, 3.52 mg of riboflavin, 14.1 mg of pantothenic acid, 28.2 mg of niacin, 140 mg of choline, 21.1 mcg of vitamin B_{12}, .11 g of chlortetracycline, .11 g of sulfamethazine and 55 mg of penicillin.

TABLE 2

PROXIMATE CHEMICAL ANALYSIS OF DIETS (%) [a]

	Treatment						
	1	2	3	4	5	6	7
Dry matter	90.95	91.12	90.98	91.18	91.28	91.00	89.54
Protein (as fed)	9.99	10.16	10.28	10.21	10.38	10.32	17.62
Protein (dry matter basis)	10.99	11.15	11.29	11.20	11.37	11.34	19.68
Ether extract (as fed)	4.14	4.37	4.01	4.10	4.03	4.64	3.08
Ether extract (dry matter)	4.55	4.80	4.41	4.50	4.41	5.10	3.43
Ash (as fed)	3.82	4.06	3.83	3.93	4.05	4.09	4.83
Ash (dry matter)	4.19	4.45	4.19	4.31	4.44	4.49	5.40

[a] A.O.A.C. (1970)

TABLE 3

ESSENTIAL AMINO ACID CONTENT OF DIETS (%)
(Calculated)

Amino Acid	Diet 1	2	3	4	5	6	7[a]
Lysine	.40	.80	.80	.80	.80	.80	.96
Methionine	.15	.15	.40	.40	.40	.40	.23
Tryptophan[a]	.12	.12	.12	.15	.15	.15	.23
Threonine	.34	.34	.34	.34	.42	.42	.71
Isoleucine	.35	.35	.35	.35	.35	.43	.99
Arginine	.60	.60	.60	.60	.60	.60	1.18
Histidine	.30	.30	.30	.30	.30	.30	.42
Leucine	.85	.85	.85	.85	.85	.85	1.61
Phenylalanine	.45	.45	.45	.45	.45	.45	.91
Valine	.50	.50	.50	.50	.50	.50	.90

[a] Estimated values.

TABLE 4

ESSENTIAL AMINO ACID CONTENT OF DIETS (%)
(Chemical Analysis)

Amino Acid	Diet 1	2	3	4	5	6	7
Lysine	.38	.77	.77	.77	.77	.77	.91
Methionine	.18	.18	.43	.43	.43	.43	.24
Threonine	.36	.36	.36	.44	.44	.44	.78
Isoleucine	.33	.33	.33	.33	.41	.41	.78
Arginine	.61	.61	.61	.61	.61	.61	.85
Histidine	.28	.28	.28	.28	.28	.27	.77
Leucine	.93	.93	.93	.93	.93	.93	1.76
Phenylalanine	.45	.45	.45	.45	.45	.45	1.02
Valine	.50	.50	.50	.50	.50	.50	.88

consume feed at will for 1 hour and then fasted for an additional 5 hours before samples were taken. Blood was drawn into a 20 ml glass syringe by a 16 gauge needle approximately 10 cm in length. Needle and syringe were consecutively rinsed with distilled water and 10% sodium citrate to prevent contamination and clotting. Samples were placed in 15 ml conical glass centrifuge tubes containing 0.1 ml 10% sodium citrate. These tubes were inverted several times for adequate mixing and then taken to the laboratory for centrifugation. Plasma was extracted and frozen in plastic tubes for later analysis. Plasma samples were prepared according to Spackman (1962) and placed on the Beckman Model 120 B Amino Acid Analyzer. An equal volume of plasma from each of the two barrows per pen and an equal volume of plasma from each of the two gilts per pen were pooled during sample preparations. Sample numbers were therefore reduced to 42 instead of 84 and results obtained were amino acid means for two barrows and for two gilts in each pen.

Data were analyzed statistically by the least squares analysis of variance outlined by Steel and Torrie (1960). When significant differences were obtained from a given set of data, Tukey's "w" procedure was used to determine which treatments were significantly different.

RESULTS AND DISCUSSION

Growth Performance

A summary of the growth performance data is presented in table 5 and the analysis of variance tables for average daily gain, feed consumption and feed efficiency are shown in tables 6, 7 and 8, respectively. Treatment means for average daily gain increased in all cases as amino acids were successively supplemented to the diet. Average daily gains were 0.33, 0.39, 0.42, 0.46, 0.53, 0.54 and 0.58 kg for diets 1 through 7, respectively. Treatment effect was significant ($P<.01$) for average daily gain. Pigs fed diet 7 gained significantly ($P<.05$) faster than pigs fed diets 1, 2 and 3 while pigs fed diets 5 and 6 gained significantly ($P<.05$) faster than those fed diet 1. Three of the four pigs fed diet 7 in replication 3 gained considerably slower than pigs in the other replicates and appeared to lack vigor and thriftiness. This resulted in lower average daily gains than would be expected for pigs fed a 18% protein corn-soybean meal diet. Rate of gain generally increased with time as the experiment progressed. Average daily gains for barrows and gilts were 0.48 kg and 0.45 kg, respectively.

Treatments did not significantly ($P<.05$) affect feed consumption, although there was a noticeable difference in consumption between the pigs fed diets 1, 2 and 3 and those fed diets 4, 5 and 6. The latter three diets were supplemented with tryptophan. Average daily feed consumption was 1.29, 1.31, 1.35, 1.51, 1.47, 1.53 and 1.44 kg for diets 1 through 7, respectively. Pigs fed diet 7 in replication 3

TABLE 5

EFFECT OF AMINO ACID SUPPLEMENTATION OF OPAQUE-2 CORN ON GROWTH PERFORMANCE OF GROWING PIGS

Treatment	1	2	3	4	5	6	7
Number of pigs[a]	12	12	12	12	12	12	12
Avg initial wt, kg	12.92	12.95	12.88	12.84	12.92	12.88	12.88
Avg final wt, kg	24.47	26.74	27.69	29.02	31.59	31.82	33.30
Avg daily gain, kg							
Rep 1	.30	.40	.40	.47	.53	.56	.65
Rep 2	.39	.36	.48	.50	.57	.50	.66
Rep 3	.30	.41	.39	.41	.50	.56	.44
Avg	.33[b]	.39[b,c]	.42[b,c]	.46[b,c,d]	.53[c,d]	.54[c,d]	.58[d]
Avg daily feed, kg							
Rep 1	1.21	1.30	1.34	1.47	1.48	1.65	1.61
Rep 2	1.50	1.32	1.50	1.66	1.60	1.46	1.61
Rep 3	1.17	1.32	1.23	1.40	1.35	1.48	1.11
Avg	1.29	1.31	1.35	1.51	1.47	1.53	1.44
Avg feed/gain							
Rep 1	3.96	3.23	3.33	3.15	2.77	2.95	2.48
Rep 2	3.87	3.64	3.12	3.31	2.78	2.89	2.44
Rep 3	3.92	3.18	3.18	3.38	2.72	2.66	2.52
Avg	3.92[b]	3.35[c]	3.21[c,d]	3.28[c]	2.76[e]	2.83[d,e]	2.48[e]

[a] Three replicated lots of four pigs each.

[b,c,d,e] Means on the same line without a common superscript were significantly different ($P<.05$).

TABLE 6

ANALYSIS OF VARIANCE FOR AVERAGE DAILY GAIN

Source of Variation	df	Mean Squares
Mean	1	88.6013
Treatment	6	.4796**
Replication	2	.1556*
Sex	1	.0635
T x R	12	.0561
T x S	6	.0783
R x S	2	.1919*
T x R x S	12	.0403
Error	42	.0384

* (P<.05)

** (P<.01)

TABLE 7

ANALYSIS OF VARIANCE FOR FEED CONSUMPTION

Source of Variation	df	Mean Squares
Total	21	
Reduction	9	22.8875
Mu	1	204.2976
Treatment	2	.1346
Replication	6	.4413**
Residual	12	.0682

** (P<.01)

TABLE 8

ANALYSIS OF VARIANCE FOR FEED EFFICIENCY

Source of Variation	df	Mean Squares
Total	21	
Reduction	9	23.1362
Mu	1	204.1729
Treatment	6	.6725*
Replication	2	.0088
Residual	12	.0181

* (P<.05)

consumed considerably less feed than pigs fed diet 7 in replications 1 and 2.

Feed efficiency was significantly (P<.05) affected by treatment. Required units of feed per unit of gain were 3.92, 3.35, 3.21, 3.28, 2.76, 2.85 and 2.48 for diets 1 through 7, respectively. Largest improvements in feed efficiency were noted with lysine supplementation between diets 1 and 2, and threonine supplementation between diets 4 and 5.

The increasing growth rates from diets 1 to 7 would indicate that limiting amino acids were added and therefore diets were better balanced and higher in amino acid content. Diet 7 was formulated to be well balanced and sufficient in amino acid content (except methionine) according to N.R.C. (1968) recommendations. N.R.C. (1968) recommends 18% protein diets for pigs 10 to 20 kg in weight and 16% protein diets for pigs which weigh 20 to 35 kilograms. Since diet 7 contained 18% protein of acceptable amino acid balance it should have been adequate to support maximum gains and feed efficiency during the entire experiment.

Opaque-2 corn is most limiting in lysine as shown by lysine supplementation of opaque-2 corn which resulted in considerably higher average daily gains and improved feed efficiency of pigs fed diet 2. The lysine content of the opaque-2 corn used in this experiment was 0.40% which is within the range of 0.37% to 0.45% lysine reported in different varieties of opaque-2 corn by Marroquin et al. (1973). These levels are considerably lower than the recommended dietary lysine requirement for weanling pigs by McWard et al. (1959), Hutchinson et al. (1957) and N.R.C. (1968).

Methionine supplementation resulted in improved gains and feed efficiency in replicate 2, however, replications 1 and 3 showed no response. The large increase in gain of pigs fed diet 3 in replication 2 was thought to be due to the lysine supplementation and not the methionine supplementation itself. Daily gains for the pigs fed diet 2 of replication 2 were especially low and did not respond to the lysine supplementation. Little or no response to methionine supplementation of opaque-2 corn was found by Oestemer et al. (1970) when using opaque-2 corn as low as 0.227% methionine plus cystine. Methionine levels in opaque-2 corn have been reported to range from 0.11% (Veum, et al., 1973) to 0.15% in this study. Recommendation of 0.5% methionine (N.R.C., 1968) for pigs weighing 20 to 35 kg is apparently too high, since this value is impossible to achieve in a practical corn-soybean meal diet of recommended protein. Acker, Catron and Hays (1959) and Wahlstrom and Libal (1973) supplemented 14% protein normal corn-soybean meal diets with methionine and found no significant improvement in performance. The data from this experiment would suggest methionine is not deficient in opaque-2 corn diets for young pigs.

Pigs fed diet 4, which was supplemented with tryptophan in addition to lysine and methionine showed relatively large increases in gain and feed consumption, again indicating an improvement in nutrient content of the diet. Estimated tryptophan level of opaque-2 corn in this study was 0.12%. N.R.C. (1968) recommends only 0.13% tryptophan for pigs weighing 20 to 35 kg and 0.18% for pigs weighing 5 to 10 kilograms. Interpolation of this data would suggest that pigs of the initial weight used in this experiment should need about 0.16% tryptophan. Diet 4 contained 0.15% tryptophan assuming 50% availability of DL-tryptophan. It has generally been assumed that only the L-isomer of tryptophan is available. Baker et al. (1971), however, reported availability of D-tryptophan as 60% and DL-tryptophan as 80% availability. If this is the case, diet 4 contained 0.16% available tryptophan.

Substantial improvement in gain, feed consumption and feed efficiency were obtained when pigs were fed diet 5 which was supplemented with threonine. The improvement in daily gain was the largest among all the amino acid supplementations. Daily gains increased from 0.46 kg when pigs were fed diet 4 to 0.53 kg for pigs fed diet 5. Diet 4 was calculated to contain 0.34% threonine and diet 5 contained 0.42% threonine. The latter figure is still slightly below the N.R.C. (1968) recommendation of 0.45% threonine for pigs from 20 to 35 kilograms. Beeson et al. (1953) recommended 0.47% threonine for weanling pigs, while Mitchell et al. (1968a) showed data which indicated a requirement of 0.6% threonine for pigs of similar weight.

There was little or no response to isoleucine supplementation in diet 6. Calculated isoleucine contents of diets 5 and 6 were 0.35% and 0.43%, respectively. N.R.C. (1968) recommends 0.76% and 0.50% isoleucine for pigs weighing 5 to 10 kg and 20 to 35 kg, respectively. Brinegar et al. (1950), Becker et al. (1957), Becker et al. (1963) and Oestemer et al. (1973) also recommended higher levels of isoleucine than were present in diet 6. The results from this experiment are therefore in disagreement with the majority of other reports since we found no performance improvement upon supplementation of the 0.35% isoleucine diet. One possibility is that there is another amino acid that is more limiting than isoleucine in this diet and which must be supplied before performance is improved.

The superior performance of pigs fed the 18% protein diet would indicate one or both of the following; other amino acids are deficient in the opaque-2 corn or suboptimum levels of one or more of the supplementary amino acids were used.

Plasma Free Amino Acids

A summary of the plasma amino acids are presented in table 9. Analysis of variance tables of the fifteen amino acids analyzed are presented in table 10. Analysis of variance tables are not presented for threonine and serine for reasons explained in table 9. Significant ($P<.05$ or $P<.01$) treatment effects were noted for eight amino acids. Of these eight, lysine, isoleucine and valine are essential amino acids while cystine, glycine, glutamic acid, proline and alanine are non-essential.

Plasma lysine levels were significantly ($P<.05$) affected by treatment. Although not significant at all levels, plasma lysine levels were notably higher for lysine supplemented diets than for diet 1 which was not supplemented with lysine. Plasma isoleucine levels were significantly ($P<.01$) affected by treatment. A large increase was noted upon isoleucine supplementation of the diet. Pigs fed diet 5 without supplemented isoleucine had 0.87 mg/100 ml plasma isoleucine and pigs fed the isoleucine-supplemented diet 6 had 1.41 mg/100 ml plasma isoleucine. The third and final essential amino acid whose plasma level was significantly ($P<.05$) affected by treatment was valine. Pigs on diets 1 to 6 had plasma valine levels ranging from 2.34 to 2.58 mg/100 ml while pigs on diet 7, the normal corn-soybean meal diet, had plasma valine levels of 3.59 mg/100 ml.

Treatment effects were highly significant ($P<.01$) for the non-essential amino acids cystine, glycine and glutamic acid, and significant ($P<.05$) for proline and alanine. It is interesting to

TABLE 9

PLASMA FREE AMINO ACID LEVELS (mg/100ml)

	Treatment						
	1	2	3	4	5	6	7
Lysine	2.59^{a}	4.51^{a,b}	5.53^{b}	6.30^{b}	4.45^{a,b}	3.97^{a,b}	5.26^{a,b}
Histidine	2.12	2.03	1.93	2.02	1.98	2.09	1.95
Ammonia	.49	.55	.64	.64	.59	.72	.71
Arginine	2.03	1.71	2.28	2.28	1.75	2.13	1.97
Aspartic Acid	.16	.14	.13	.15	.17	.25	.20
Glutamic Acid	4.13^{a}	4.64^{a,b}	4.00^{a}	3.78^{a}	4.89^{a,b}	6.45^{b}	3.47^{a}
Proline	3.77^{a}	3.44^{a,b}	3.26^{a,b}	2.97^{a,b}	3.41^{a,b}	3.52^{a,b}	2.61^{b}
Glycine	6.24^{a}	9.23^{b,c}	8.87^{a,b,c}	8.54^{a,b,c}	11.27^{c}	9.47^{b,c}	8.16^{a,b}
Alanine	5.68^{a}	6.20^{a}	6.12^{a}	5.24^{a,b}	6.05^{a}	5.83^{a}	3.15^{b}
Half Cystine	.19^{a,b}	.13^{a}	.34^{a,b,c}	.36^{a,b,c}	.55^{b,c}	.66^{c}	.22^{a,b}
Valine	2.51^{a,b}	2.58^{a,b}	2.38^{a,b}	2.44^{a,b}	2.34^{b}	2.44^{a,b}	3.59^{a}
Methionine	.53	.50	.62	.68	.69	.70	.57
Isoleucine	1.00^{a,b}	.93^{a,b}	.84^{b}	.91^{a,b}	.87^{b}	1.41^{a,b}	1.48^{a}
Leucine	2.24	2.58	2.26	2.32	2.72	2.91	2.76
Tyrosine	1.50	1.27	1.32	1.34	1.30	1.28	1.77
Phenylalanine	1.34	1.24	1.13	1.11	1.14	1.19	1.44
Threonine[d]	3.06	.81	.81	1.09	1.39	1.86	.91
Serine[d]	2.47	2.18	1.87	2.02	2.35	1.75	1.67

a,b,c Means on the same line without a common superscript were significantly different ($P<.05$).

d Threonine and serine values were difficult to interpolate from the amino acid graphs due to interference by other compounds. Values presented here were obtained by pooling all samples within a treatment, hydrolyzing these pooled samples mildly to eliminate interferring compounds and placing a corresponding volume of the pooled sample on the column. Statistical analysis was therefore not achieved on these two amino acids.

TABLE 10

ANALYSIS OF VARIANCE FOR PLASMA AMINO ACIDS

Source of Variation	df	Lysine Mean Square	Glycine Mean Square	Histidine Mean Square	Ammonia Mean Square	Argininine Mean Square	Aspartic Acid Mean Square
Total	42						
Reduction	30	34.6519	115.8714	5.9672	.7163	6.2011	.0486
Mu	1	910.8440	3269.9273	170.6907	16.1944	171.5789	1.2172
Treatment	6	8.6251*	13.7753**	.0298	.0426	.3178	.0101
Replication	2	21.0402**	35.6579**	1.7620**	1.7651**	1.8117*	.0095*
Sex	1	.8093	14.3501	1.0029	.0014	.2736	.0004
T x R	12	2.0377	1.9087	.1823	.0942**	.3737	.0124**
T x S	6	1.4063	.8648	.0975	.0596*	.3885	.0015
R x S	2	.5915	4.9026	.4232	.0093	.9184	.0019
Residual	12	1.2049	1.7184	.1586	.0132	.3166	.0023

* (P<.05)

** (P<.01)

TABLE 10 (CONTINUED)

Source of Variation	df	Glutamic Acid Mean Square	Proline Mean Square	Alanine Mean Square	Half Cystine Mean Square	Valine Mean Square
Total	42					
Reduction	30	31.0012	15.6059	44.4362	.2417	10.0960
Mu	1	842.1506	451.9840	1254.2722	5.0371	286.4215
Treatment	6	5.9190**	.8875*	6.8960*	.2293**	1.1616*
Replication	2	18.3447**	2.2542*	2.0571	.1493**	2.0298**
Sex	1	2.2172	.7150	.0933	.0030	.2058
T x R	12	.8243	.2401	1.4471	.0367	.3628
T x S	6	.3039	.3744	1.3008	.0138	.1293
R x S	2	.8751	.2590	4.0310	.0060	.0469
Residual	12	.9583	.4662	1.4252	.0175	.1968

* $(P<.05)$

** $(P<.01)$

TABLE 10 (CONTINUED)

Source of Variation	df	Methionine Mean Square	Isoleucine Mean Square	Leucine Mean Square	Tyrosine Mean Square	Phenylalanine Mean Square
Total	42					
Reduction	30	.5619	1.7506	9.3944	2.9206	2.2321
Mu	1	15.8117	47.3185	270.4594	81.9285	63.2224
Treatment	6	.0388	.4276**	.4358	.2018	.0928
Replication	2	.1697**	.7612**	2.3332**	1.1323**	.8541**
Sex	1	.0000	.0064	.0028	.0202	.0293*
T x R	12	.0274	.0793	.2557	.1173	.0979
T x S	6	.0233	.0249	.1686	.1140	.0450
R x S	2	.0014	.0026	.0052	.0517	.0003
Residual	12	.0123	.0531	.1902	.0721	.0486

* ($P<.05$)

** ($P<.01$)

note the rise in plasma half cystine level in pigs from diet 2 (which is not supplemented with methionine) to diet 3 (which is supplemented with methionine). It has been reported by Becker et al. (1955a) that cystine can provide up to 40% of the methionine requirement. Apparently cystine was being utilized to supply the methionine requirement until additional methionine was added. Glycine levels followed no particular pattern with diet 1 pigs exhibiting the lowest plasma glycine levels. Plasma levels of glutamic acid, proline and alanine are the lowest for pigs which are on diet 7 which contains the highest total amino acid content.

According to Zimmerman and Scott (1965) there is a definite relationship between plasma amino acid levels and the amino acid adequacy of the diet. Such was the case with this experiment since lysine, methionine, threonine and isoleucine plasma levels increased with the supplementations of the four respective amino acids.

The increase in plasma lysine with dietary lysine supplementation is in agreement with Morrison et al. (1961), McLaughlin and Illman (1967), Mitchell et al. (1968b) and Harker et al. (1968). However, as additional amino acids are supplemented we see a gradual increase and then decrease in plasma lysine level despite similar total lysine level in the diet. This may indicate an initial problem with amino acid imbalance resulting in higher plasma lysine levels and then an improvement in amino acid balance upon threonine and isoleucine supplementation. This improved balance would result in higher tissue utilization of lysine and therefore a lower level in the plasma. The results with plasma lysine level in this experiment would indicate that the use of plasma lysine levels to determine a lysine deficiency in a diet is limited if other amino acids are limiting, since lysine levels in the plasma varied when the same level of lysine was fed with different levels of other amino acids.

Isoleucine supplementation of the diet resulted in plasma isoleucine levels rising from 0.87 mg/100 ml to 1.41 mg/100 ml. Diet 7 contained over twice the calculated level of isoleucine as diet 6, yet pigs fed diet 7 showed plasma isoleucine levels of only 1.48 mg/100 ml. Since diet 7 should have been more than adequate in isoleucine by N.R.C. (1968) standards, the similarity in plasma isoleucine levels between pigs on diet 6 and diet 7 would indicate that diet 6 was adequate in isoleucine.

Although not significant, pigs from the methionine supplemented diets 3 to 6 had higher plasma methionine levels than pigs on the unsupplemented diets 1 and 2. In addition, pigs on diets 3 to 6 showed higher plasma methionine levels than pigs on diet 7. Cystine, which can replace a portion of the methionine requirement (N.R.C., 1968), also rose as methionine was supplemented, indicating a decrease or elimination of this replacement function of cystine for methionine. Both methionine and half cystine plasma levels are higher in diet 6 than in the 18% protein diet 7 (which gave considerably higher gains than diet 6) indicating that the calculated 0.4% dietary methionine is more than adequate for pigs of this weight. This is in disagreement with N.R.C. (1968) and Curtin *et al.* (1952a) who recommend higher levels of methionine. However, Oestemer et al. (1970) found that methionine supplementation of opaque-2 corn with methionine plus cystine levels as low as 0.227% gave no significant improvement in performance.

SUMMARY

Eighty-four crossbred pigs weighing approximately 13 kg initially were used in three replications of seven treatments to study the amino acid deficiencies of opaque-2 corn. Successive supplementations of lysine, methionine, tryptophan, threonine and isoleucine to opaque-2 corn were made to formulate diets 1 through 6, with diet 7 being a normal corn-soybean meal diet containing 18% protein.

Treatments significantly affected rate of gain ($P<.01$) and feed efficiency ($P<.05$). Supplementing the opaque-2 corn with 0.4% L-lysine improved gain and efficiency considerably. Pigs fed diet 1 gained 0.33 kg and pigs fed the lysine supplemented diet 2 gained 0.39 kg daily. Feed/gain, which was significantly ($P<.05$) improved by lysine supplementation, was 3.92 and 3.35, for diets 1 and 2, respectively. Plasma lysine levels were significantly ($P<.05$) affected by treatment. Pigs which were fed lysine supplemented diets had plasma lysine levels ranging from 3.97 mg/100 ml to 6.30 mg/100 ml, while pigs fed diet 1 which was not supplemented had 2.59 mg/100 ml plasma.

Methionine supplementation data were inconsistent. Improved performance was noted only in replication 2, while rate of gain and feed efficiency were unaffected in replications 1 and 3. Treatment effects were not significant for plasma methionine levels, but half cystine levels were significantly ($P<.01$) affected by treatments. Pigs fed the methionine supplemented diets had higher plasma methionine and half cystine levels than pigs fed diets 1 and 2 which were not supplemented with methionine.

Increased feed consumption and average daily gain were noted when pigs were fed tryptophan supplemented diets. Average daily gain increased from 0.42 kg in diet 3 to 0.46 kg in diet 4 which was supplemented with tryptophan. Daily feed consumption rose from 1.35 kg in diet 3 to 1.51 kg in diet 4.

Pigs fed diets supplemented with threonine showed marked improvement in average daily gain and feed efficiency. Average daily gain increased 0.07 kg and feed/gain decreased by 0.52 kg for pigs fed the threonine supplemented diet 5 compared to the non-threonine supplemented diet 4. Threonine plasma levels dropped considerably upon lysine supplementation in diet 2 and increased somewhat upon threonine supplementation.

Growth performance was not appreciably affected by isoleucine addition in diet 6. Treatment effects were significant ($P<.01$) for plasma isoleucine levels, with plasma levels of pigs fed diet 6 approaching those of pigs fed diet 7, which was considerably higher in isoleucine.

Plasma levels of the essential amino acid valine were significantly ($P<.05$) affected by treatment. Pigs fed diet 7 had considerably higher levels of plasma valine than those fed diets 1 through 6. Non-essential amino acid levels in the plasma were also affected by treatments. The non-essential amino acids significantly ($P<.05$ or $P<.01$) affected were glycine, glutamic acid, proline and alanine. The plasma levels of the latter three amino acids were lowest for pigs fed diet 7.

From the data presented herein it appears that lysine, tryptophan and threonine are the three most limiting amino acids in the opaque-2 corn used in this experiment. Methionine and isoleucine did not appear to be deficient in the opaque-2 corn diet.

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